



UNIVERSITI PUTRA MALAYSIA

**NITROGEN FIXATION AND PLANT GROWTH ENHANCEMENT BY
BENEFICIAL RHIZOBACTERIA IN ASSOCIATION WITH OIL PALM
SEEDLINGS**

AMIR HAMZAH BIN AHMAD GHAZALI

FP 2001 1

**NITROGEN FIXATION AND PLANT GROWTH ENHANCEMENT BY
BENEFICIAL RHIZOBACTERIA IN ASSOCIATION WITH OIL PALM
SEEDLINGS**

By

AMIR HAMZAH BIN AHMAD GHAZALI

**Thesis Submitted in Fulfilment of the Requirement for the
Degree of Doctor of Philosophy in the Faculty of Agriculture,
Universiti Putra Malaysia**

December 2001



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

**NITROGEN FIXATION AND PLANT GROWTH ENHANCEMENT BY
BENEFICIAL RHIZOBACTERIA IN ASSOCIATION WITH OIL PALM
SEEDLINGS**

By

AMIR HAMZAH BIN AHMAD GHAZALI

December 2001

Chairman : Associate Professor Dr. Zulkifli Hj. Shamsuddin

Faculty : Agriculture

Nitrogen fertilizer is the most expensive nutrient input in oil palm production with average total estimated cost amounting to RM 470 million year⁻¹. Rapid losses of N fertilizer through leaching further increases the cost on palm oil production and makes the sector less profitable. Recently there is a new finding about an associated N₂ fixing rhizobacteria which could be applied to the oil palm seedling production sector. The beneficial rhizobacteria has been reported to be efficient in N₂ fixation and stimulates plant growth, thus could be used as a biofertilizer and bioenhancer, respectively, for various leguminous and non-leguminous crops. Therefore, a series of experiments were carried out to estimate the N₂ fixing capacity of the rhizobacteria and to observe the effects of inoculation on growth and development of the associated oil palm seedlings.

Results from the initial experiment indicated that *Azospirillum* could contribute 40% of the total N requirement of oil palm plantlets through N₂ fixation process, stimulated total dry matter accumulation and root growth, stimulated uptake and higher concentration of N and K and increased the host photosynthetic rates compared to the

control, after 120 days of planting (D₁₂₀). The subsequent pot experiment (glasshouse experiment; harvested after 130, 260 and 390 days of planting (D₁₃₀, D₂₆₀ and D₃₉₀)) demonstrated the potential of *Azospirillum brasilense* Sp 7 and locally isolated *Bacillus sphaericus* UPMB 10 and *Bacillus subtilis* UPMB 13 as a biofertilizer for oil palm seedlings through N₂ fixation (25-50% Nd_{fa}; Nitrogen derived from atmosphere) and stimulated higher concentration of essential nutrients and their accumulation especially N, P and K. The inocula also enhanced the development of roots (root dry weight, volume, primary root numbers and high R/S ratio) and tops (total dry matter, top dry weight, chlorophyll content, leaf area, plant height and stem diameter) of the host plants. Generally, effects of the inoculation at two monthly intervals were more promising during the earlier growth stage (D₁₃₀-D₂₆₀) with similar or better response compared to the control with complete N_i fertilization (Sp 7 k +N_i) especially for all root growth observations, top dry weight and total dry matter of the host plants. However, this improvement in plant growth was less than the control with complete N_i fertilization after an intentionally prolonged nursery phase, D₃₉₀. A duplicated experiment as above was carried out in the field nursery station, FELDA Bukit Mendi, Pahang. The inoculation process showed positive response in enhancing higher concentration and accumulation of N, P and K for the host plants especially at D₁₃₀, enhanced root growth (root dry weight, volume, primary root number, and R/S ratio) until end of the growth stages (D₃₉₀) and top growth which includes total dry matter, top dry weight and chlorophyll content of the host plants (until D₂₆₀).

The *in vitro* N₂ fixation study using (¹⁵N) dilution method clearly showed an increase in N₂ fixation capacity (67% Nd_{fa}) for the Sp 7 strain associated with tissue-cultured oil palm plantlets, at D₅₆. Similarly, the locally isolated UPMB 10 and UPMB 13

strains could contribute up to 55% Ndfa. The inoculation process also caused significant increase in total N and higher leaf chlorophyll content of the host plants, although the response was still less compared to the control with complete N_i fertilization. The enhancement in root growth and dry matter production were observed to produce comparable effects to those supplied with complete inorganic-N. All the inocula tested could successfully colonize the root-surface and improve the host plant growth.

The above findings provided evidence that *Azospirillum* and locally isolated *Bacillus* spp. are potentially effective biofertilizers and bioenhancers for sustainable oil palm seedling production. However, more frequent inoculation and higher inoculum size will be required to maintain the inoculum population in the soil especially during a prolonged growth phase of the host plants (D_{390}) under an extended nursery condition.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENGIKATAN NITROGEN DAN PENINGKATAN TUMBESARAN POKOK
OLEH RHIZOBAKTERIA BERFAEDAH DALAM ASSOSIASI DENGAN ANAK
BENIH KELAPA SAWIT**

Oleh

AMIR HAMZAH BIN AHMAD GHAZALI

Disember 2001

Pengerusi : Profesor Madya Dr. Zulkifli Hj. Shamsuddin

Fakulti : Pertanian

Baja nitrogen merupakan sumber nutrien yang paling mahal bagi perusahaan kelapa sawit dan melibatkan purata kos hampir RM 470 juta tahun⁻¹. Kehilangan baja nitrogen melalui proses larut-lesap merupakan antara faktor yang mempengaruhi peningkatan kos pengeluaran kelapa sawit dan seterusnya mengurangkan profit sektor ini. Akhir-akhir ini terdapat penemuan baru berkenaan rhizobakteria pengikat N₂ yang berpotensi untuk diaplikasikan kepada sektor pengeluaran anak benih kelapa sawit. Rhizobakteria berfaedah ini di laporkan amat berkesan bagi proses pengikatan N₂ (bio-baja) dan penggalak tumbesaran pokok (bio-penggalak) bagi beberapa jenis perumah kekacang dan bukan kekacang. Oleh yang demikian, beberapa siri eksperimen telah dijalankan untuk menganggarkan keupayaan pengikatan N₂ dan melihat kesan inokulasi rhizobakteria tersebut terhadap pertumbuhan dan perkembangan anak benih kelapa sawit.

Keputusan daripada kajian awal menunjukkan bahawa proses inokulasi *Azospirillum* dapat menyumbang sehingga 40% daripada jumlah keseluruhan N yang

diperlukan oleh anak benih kelapa sawit melalui proses pengikatan N_2 , meningkatkan berat kering total pokok dan pertumbuhan akar, meningkatkan kadar pengambilan N dan K dan peningkatan kadar fotosintesis perumah berbanding kawalan selepas ditanam selama 120 hari (D_{120}). Kajian berpasu berikutnya (rumah kaca; pengumpulan data pada 130, 260 dan 390 hari selepas penanaman (D_{130} , D_{260} and D_{390})) menunjukkan potensi *Azospirillum brasilense* Sp 7, isolat tempatan *Bacillus sphaericus* UPMB 10 dan *Bacillus subtilis* UPMB 13 sebagai bio-baja untuk anak benih kelapa sawit berdasarkan 25-50% kadar pengikatan N_2 (%Ndfa) dan meningkatkan kadar pengambilan dan pengumpulan nutrien penting terutamanya N, P dan K. Inokulum yang digunakan juga dapat menggalakkan perkembangan akar (berat kering akar, isipadu, bilangan akar primer dan nisbah akar/pucuk 'R/S ratio' yang lebih tinggi) dan pucuk (berat kering total, berat kering pucuk, kandungan klorofil, keluasan daun, ketinggian pokok dan diameter batang) perumah. Secara amnya, kesan inokulasi adalah lebih baik pada peringkat awal pertumbuhan (D_{130} - D_{260}) selepas beberapa siri proses inokulasi pada kekerapan setiap dua bulan dengan kesan yang setara dan lebih baik berbanding kawalan dengan pembajaan penuh N (Sp 7 k + N_i) terutamanya bagi perkembangan pertumbuhan akar pokok, berat kering pucuk dan berat kering total perumah. Walau bagaimanapun kesan positif tersebut mulai manurun berbanding kawalan dengan pembajaan penuh N_i selepas fasa penanaman di tapak semaian dilanjutkan sehingga D_{390} . Satu kajian yang sama seperti kajian yang di atas telah dijalankan di ladang tapak semaian kelapa sawit, FELDA Bukit Mendi, Pahang. Proses inokulasi yang dijalankan telah menunjukkan kesan positif bagi pengumpulan dan pengambilan N, P dan K untuk perumah terutamanya pada D_{130} , meningkatkan pertumbuhan akar (berat kering akar, isipadu, bilangan akar primer dan nisbah akar/pucuk 'R/S ratio' yang lebih tinggi) sehingga D_{390} . Pertumbuhan pucuk

perumah juga meningkat dengan proses inokulasi tersebut terutamanya jumlah berat kering, berat kering pucuk dan kandungan klorofil perumah (sehingga D₂₆₀).

Kajian berikutnya, melibatkan kajian pengikatan N₂ *in vitro* (¹⁵N isotope dilution method') yang menunjukkan keupayaan pengikatan N₂ sehingga 67% Ndfa bagi inokulasi Sp 7 ke atas tisu kultur kelapa sawit pada D₅₆. Sementara inokulasi isolat tempatan strain UPMB 10 dan UPMB 13 dapat menyumbang sehingga 55% Ndfa. Proses inokulasi tersebut juga dapat meningkatkan kandungan N total dan klorofil perumah walaupun kesannya masih rendah berbanding kawalan dengan pembajaan penuh N_i. Peningkatan pertumbuhan akar dan berat kering perumah adalah sama seperti kawalan dengan pembajaan penuh N_i. Kesemua inokula yang dikaji menunjukkan keupayaan kolonisasi permukaan akar tisu kultur kelapa sawit yang seterusnya membantu pertumbuhan perumahnya.

Penemuan di atas dapat membuktikan bahawa *Azospirillum* dan isolat tempatan *Bacillus* spp. mempunyai potensi yang baik sebagai penggalak (bio-baja dan bio-penggalak) pertumbuhan lestari anak benih kelapa sawit. Kekerapan inokulasi yang lebih tinggi dan saiz inokulum yang lebih besar adalah amat diperlukan untuk mengekalkan populasi inokula yang tinggi di dalam tanah untuk tempoh pertumbuhan perumah yang lebih lama (D₃₉₀) di tapak semaian.

ACKNOWLEDGEMENTS

My sincere appreciation is extended to Associate Professor Dr. Zulkifli Hj. Shamsuddin, Dr. Halimi Mohd. Saud, Associate Professor Dr. Mohd. Fauzi Ramlan and Professor Dr. Marziah Mahmood for their invaluable guidance, advice and comments during the planning and execution of this study and in the preparation of this manuscript.

Special appreciation is also extended to Tn. Hj. Husain Menggong (FELDA) for providing the planting materials (oil palm seedlings) and experimental sites at FELDA Bukit Mendi, Pahang for this study and Mr. Zamzuri Ishak (MPOB) and Ms. Noraini (MPOB) for the tissue-cultured oil palm plantlets. I really appreciated the help and co-operation from Professor Dr. Zaharah Abd. Rahman (UPM), Dr. Philip M. Chalk (IAEA, Vienna), Dr. Norimah Yusuf (MINT), Dr. Zainab Hamzah (MRB), Dr. Khairuddin A. Rahim (MINT), Dr. Asiah Ahmad (MINT), En. Ahmad Sahali Mardi (MINT) and Mrs. Latifah Nordin (MINT) in conducting the ^{15}N experiments and sample analyses.

Gratitude is also expressed to all lecturers at Department of Land Management, UPM, Mr. Kamarudin Yusof, Mrs. Zarinah Basir, Mr. Dzulkifli Duaji, Mr. Alias Tahar, Mr. Junaidi Jaafar, Mr. Rahim Utar, Mr. Jamil Othman, Mr. Mazlan Bangi, Mr. Azhar Othman, Mr. Abdul Aziz Ismail, Mrs. Sabariah Buang and my colleagues (Dr. Md. Abdul Baset Mia (BINA), Dr. Abul Hossain Molla (BSMRAU), Mr. Neo Sye Peng, Mr. Ooi Tze Chean, Ms. Khor Sock Kun, Ms. Tan Geok Hun, Ms. Premalatha Pakirisamy, Dr. Muskhazli Mustafa, Dr. Norazwadi Abd. Aziz and Dr. Jualang Azlan

Gansau (UMS)) for their continuous comments, advice and assistance throughout the study.

The research project was made possible by the financial support of IRPA fund (Enhancement in early growth of oil palm seedlings inoculated with *Azospirillum* spp. : Project No. 01-02-04-0372) and a scholarship from Universiti Sains Malaysia under Academic Staff Training Programme scheme.

I am deeply indebted to my wife *SITI INDRIATI* my sons *IKHWAN* and *IQBAL*, my parents and all my in-laws for their sacrifices and patience during the course of my graduate study.

I certify that an Examination Committee met on 29th December 2001 to conduct the final examination of Amir Hamzah Bin Ahmad Ghazali on his Doctor of Philosophy thesis entitled "Nitrogen Fixation and Plant Growth Enhancement by Beneficial Rhizobacteria in Association with Oil Palm Seedlings" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

ROSENANI ABU BAKAR, Ph.D.
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairperson)

ZULKIFLI HJ. SHAMSUDDIN, Ph.D.
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

HALIMI MOHD. SAUD, Ph.D.
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

MOHD. FAUZI RAMLAN, Ph.D.
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

MARZIAH MAHMOOD, Ph.D.
Professor
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

NANTAKORN BOONKERD, Ph.D.
Professor
Institute of Research and Development
Suranaree University of Technology
Thailand
(Independent Examiner)



AINI IDERIS, Ph.D.
Professor/Dean of Graduate School
Universiti Putra Malaysia

Date : 8 JAN 2002

This thesis submitted to the Senate Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy.



AINI IDERIS, Ph.D.

Professor/

Dean of Graduate School

Universiti Putra Malaysia

Date : **14** MAR 2002

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.


AMIR HAMZAH BIN AHMAD GHAZALI

Date : 7 January 2002

TABLE OF CONTENTS

ABSTRACT	page ii
ABSTRAK	v
ACKNOWLEDGEMENTS	viii
APPROVAL	x
DECLARATION	xii
TABLE OF CONTENTS	xiii
LIST OF TABLES	xvii
LIST OF FIGURES	xx
LIST OF APPENDIXES	xxiv
LIST OF ABBREVIATIONS	xxv

CHAPTER

1 INTRODUCTION	1
2 REVIEW OF LITERATURE	
2.1 Malaysian Palm Oil Industry	7
2.2 Plant Growth Promoting Rhizobacteria (PGPR)	8
2.2.1 <i>Azospirillum</i> spp.	10
2.2.2 <i>Bacillus</i> spp.	12
2.3 Associative Biological N ₂ Fixation (BNF) with Non-Legumes.....	13
2.3.1 Applications of ¹⁵ N Isotope Dilution Technique to Estimate N ₂ Fixation Rates	16
2.4 Effects of Rhizobacteria Inoculation on Plant Growth and Development	19
2.4.1 The Role of N on the Photosynthetic Rates of the Inoculated Host Plants	22
2.4.2 Plant Nutrient Uptake and Accumulation	25
2.4.3 Root Colonization	26
3 GENERAL MATERIALS AND METHODS	
3.1 Soil Preparation	29
3.2 Preparation and Application of Inoculum	30
3.3 Determination of Leaf Chlorophyll Content	32
3.4 Determination of P, K, Ca and Mg Concentrations and Accumulation in Plant Sample	33
3.5 Total N Determination and Estimation of N ₂ Fixation Process	34
3.6 Statistical Analysis	37

4	EFFECTS OF <i>Azospirillum</i> INOCULATION ON N₂ FIXATION AND GROWTH OF OIL PALM PLANTLETS UNDER GLASSHOUSE CONDITIONS	
4.1	Introduction	38
4.2	Materials and Methods	39
4.3	Results	
4.3.1	¹⁵ N Isotope Dilution Analysis	47
4.3.2	Essential Nutrient Analysis.....	48
4.3.3	Plant Growth and Dry Matter Accumulation	49
4.3.4	Photosynthetic Activities of Oil Palm Plantlets	50
4.4	Discussion	53
4.5	Conclusion	59
5	ESTIMATION OF N₂ FIXATION RATES AND PLANT GROWTH PROMOTION BY RHIZOBACTERIA IN ASSOCIATION WITH OIL PALM SEEDLINGS UNDER GLASSHOUSE AND FIELD NURSERY CONDITIONS	
5.1	Introduction	60
5.2	Materials and Methods	61
5.3	Results	
5.3.1	Glasshouse Experiment	
5.3.1.1	Dilution of ¹⁵ N Isotope and Partitioning	68
5.3.1.2	Total N ₂ Fixation Rates	71
5.3.1.3	Distribution of Fixed N ₂ in Leaves, Stems and Roots of Inoculated Oil Palm Seedlings	74
5.3.1.4	Distribution of N, P, K, Ca and Mg concentrations in Different Plant Parts of Inoculated Oil Palm Seedlings	77
5.3.1.5	Total Accumulation (g plant ⁻¹) and Tissue Concentration (%) of N, P, K, Ca and Mg in Inoculated Oil Palm Seedlings	82
5.3.1.6	Root Dry Weight (g plant ⁻¹)	85
5.3.1.7	Root Volume (cm ³ plant ⁻¹)	85
5.3.1.8	Primary Root Numbers	86
5.3.1.9	Top Dry Weight (g plant ⁻¹)	86
5.3.1.10	Total Leaf Area (cm ² plant ⁻¹)	89
5.3.1.11	Leaf Chlorophyll Content	90
5.3.1.12	Plant Height (cm plant ⁻¹)	91

5.3.1.13	Stem Diameter (mm plant ⁻¹) and Leaf Number	92
5.3.1.14	Dry Matter Accumulation (g plant ⁻¹)	94
5.3.1.15	Root-Shoot Ratio Analysis	94

5.3.2 Field Experiment

5.3.2.1	Distribution of N, P, K, Ca and Mg in Different Plant Parts of Inoculated Oil Palm Seedlings	97
5.3.2.2	Total Accumulation (g plant ⁻¹) and Tissue Concentration of N, P, K, Ca and Mg	100
5.3.2.3	Root Dry Weight (g plant ⁻¹)	104
5.3.2.4	Root Volume (cm ³ plant ⁻¹)	104
5.3.2.5	Primary Root Numbers	106
5.3.2.6	Top Dry Weight (g plant ⁻¹)	106
5.3.2.7	Total Leaf Area (cm ² plant ⁻¹)	108
5.3.2.8	Leaf Chlorophyll Content	108
5.3.2.9	Stem Diameter (mm plant ⁻¹)	110
5.3.2.10	Plant Height (cm plant ⁻¹) and Leaf Number	110
5.3.2.11	Dry Matter Accumulation (g plant ⁻¹)	112
5.3.2.12	Root-Shoot Ratio Analysis	112

5.4	Discussion	115
5.5	Conclusion	125

6 N₂ FIXATION, PLANT GROWTH ENHANCEMENT AND ROOT-SURFACE COLONIZATION BY RHIZOBACTERIA IN ASSOCIATION WITH OIL PALM PLANTLETS UNDER *IN VITRO* CONDITIONS

6.1	Introduction	126
6.2	Materials and Methods	127
6.3	Results	
6.3.1	¹⁵ N Isotope Dilution and N ₂ Fixation Rates	132
6.3.2	Total N Accumulation (mg plant ⁻¹) and N concentration (%) of Inoculated Oil Palm Plantlets	133
6.3.3	Leaf Chlorophyll Content	134
6.3.4	Root Growth and Development	135
6.3.5	Total Dry Matter (mg plant ⁻¹)	138
6.3.6	Root Colonization	139
6.4	Discussion	141
6.5	Conclusion	146

7	GENERAL DISCUSSION AND CONCLUSION	147
	BIBLIOGRAPHY.....	156
	APPENDICES	172
	VITA	179

LIST OF TABLES

Table	page
3.1 Chemical properties of Selangor series soil (<i>Typic sulfic tropaquept</i>) (0-15 cm depth).	30
3.2 Growth medium for <i>Azospirillum</i> and locally isolated- <i>Bacillus</i> spp.	31
4.1 N-free basal fertilizer rate based on recommended fertilizer applications for oil palm plantlets at nursery stage.	40
4.2 Effects of <i>Azospirillum</i> inoculation on % ¹⁵ N atom excess (a.e.), estimated biologically fixed nitrogen (% Ndfa) and total N ₂ fixed in shoots of oil palm plantlets after 120 days of growth in ¹⁵ N labeled Selangor series soil.	47
4.3 Effects of <i>Azospirillum</i> inoculation on concentrations of N, P, K, Ca and Mg in shoots of oil palm plantlets after 120 days of growth in Selangor series soil.	48
4.4 Effects of <i>Azospirillum</i> inoculation on total accumulation/uptake of N, P, K, Ca and Mg for oil palm plantlets after 120 days of growth in Selangor series of soil.	49
4.5 Effects of <i>Azospirillum</i> inoculation on growth parameters of oil palm plantlets after 120 days of growth in Selangor series soil.	50
4.6 Effects of <i>Azospirillum</i> inoculation on light response characteristics of oil palm plantlets after 120 days of growth in Selangor series soil.	51
4.7 Effects of <i>Azospirillum</i> inoculation on CO ₂ response characteristics of oil palm plantlets after 120 days of growth in Selangor series soil.	52
5.1 Basal fertilizer requirement based on recommended fertilizer applications for oil palm seedlings for the first 4 months of growth in 8 kg soil (40 mL/pot).	62
5.2 Basal fertilizer rate based on recommended fertilizer applications for oil palm seedlings from month 5 – 12.	63

5.3	Partition for percentage of ^{15}N atom excess in different plant parts of inoculated oil palm seedlings at 130, 260 and 390 days after planting in Selangor series soil under glasshouse conditions.	70
5.4	Partition for percentage of N_2 derived from atmosphere (%Ndfa) in different plant parts of inoculated oil palm seedlings at 130, 260 and 390 days after planting in Selangor series soil under glasshouse conditions. (<i>Calculated based on Sp 7 k (nss) and Sp 7 k (ss) reference plants</i>)	75
5.5	Partition of total N_2 fixed (mg N plant^{-1}) in different plant parts of inoculated oil palm seedlings at 130, 260 and 390 days after planting in Selangor series soil under glasshouse conditions. (<i>Calculated based on Sp 7 k (nss) and Sp 7 k (ss) reference plants</i>)	76
5.6	Effects of rhizobacterial inoculation on partitioning of N, P, K, Ca and Mg concentrations in different plant parts of oil palm seedlings at 130 days after planting in Selangor series soil under glasshouse conditions.	78
5.7	Effects of rhizobacterial inoculation on partitioning of N, P, K, Ca and Mg concentrations in different plant parts of oil palm seedlings at 260 days after planting in Selangor series soil under glasshouse conditions.	80
5.8	Effects of rhizobacterial inoculation on partitioning of N, P, K, Ca and Mg concentrations in different plant parts of oil palm seedlings at 390 days after planting in Selangor series soil under glasshouse conditions.	81
5.9	Effects of rhizobacterial inoculation on total accumulation of N, P, K, Ca and Mg of oil palm seedlings at 130, 260 and 390 days after planting in Selangor series soil under glasshouse conditions.	83
5.10	Effects of rhizobacterial inoculation on concentrations of N, P, K, Ca and Mg of oil palm seedlings at 130, 260 and 390 days after planting in Selangor series soil under glasshouse conditions.	84
5.11	Effects of rhizobacterial inoculation on plant height (cm plant^{-1}), stem diameter (mm plant^{-1}) and leaf numbers of oil palm seedlings at 130, 260 and 390 days after planting in Selangor series soil under glasshouse conditions.	93

5.12	Effects of rhizobacterial inoculation on partitioning of N, P, K, Ca and Mg concentrations in different plant parts of oil palm seedlings at 130 days after planting in Selangor series soil under field conditions.	98
5.13	Effects of rhizobacterial inoculation on partitioning of N, P, K, Ca and Mg concentrations in different plant parts of oil palm seedlings at 260 days after planting in Selangor series soil under field conditions.	99
5.14	Effects of rhizobacterial inoculation on partitioning of N, P, K, Ca and Mg concentrations in different plant parts of oil palm seedlings at 390 days after planting in Selangor series soil under field conditions.	101
5.15	Effects of rhizobacterial inoculation on total accumulation of N, P, K, Ca and Mg of oil palm seedlings at 130, 260 and 390 days after planting in Selangor series soil under field conditions.	102
5.16	Effects of rhizobacterial inoculation on concentrations of N, P, K, Ca and Mg of oil palm seedlings at 130, 260 and 390 days after planting in Selangor series soil under field conditions.	103
5.17	Effects of rhizobacterial inoculation on stem diameter (mm plant ⁻¹), plant height (cm plant ⁻¹) and leaf number of oil palm seedlings at 130, 260 and 390 days after planting in Selangor series soil under field conditions.	111
6.1	N-free MS medium for growth of inoculated oil palm plantlets.	128
6.2	Percentage of ¹⁵ N atom excess (a.e.), N ₂ derived from atmosphere (%Nd _{fa}) and N ₂ fixed (mg N plant ⁻¹) of inoculated oil palm plantlets at D ₅₆ .	133

LIST OF FIGURES

Figure	page
3.1 Standard curve for total leaf chlorophyll content (mg chlorophyll/mg leaf fresh weight) and CM value (leaf greenness) for oil palm plantlets.	33
4.1 Selected 2 months old oil palm plantlets (Clone P149, provided by Malaysian Palm Oil Board (MPOB)).	42
4.2 Portable infrared gas analyzer LI-6200™ (Licor Inc. Nebraska, USA).	43
4.3 Measurement on light response curve of the inoculated oil palm plantlet by portable infrared gas analyzer LI-6200™.	43
4.4 Photosynthetic analysis for a) light response and b) CO ₂ response characteristics on tested palms (Henson, 1991).	45
4.5 Effects of <i>Azospirillum</i> inoculation (Sp 7 and CCM 3863) on photosynthetic rates of oil palm plantlets after 120 days of growth in Selangor series soil.	51
4.6 Effects of <i>Azospirillum</i> inoculation (Sp 7 and CCM 3863) on photosynthetic rates of oil palm plantlets after 120 days of growth in Selangor series soil.	52
5.1 (a) Oil palm seeds (DxP Yangambi) and (b) first inoculation process after 14 days of growth for both glasshouse and field experiments.	66
5.2 Applications of (a) ¹⁵ N labeled fertilizer (glasshouse experiment only), (b) N-free basal fertilizer and (c) measurement on chlorophyll meter (CM) values for leaf greenness (SPAD meter-502, MINOLTA™).	66
5.3 Buchi K314™ distillation unit (glasshouse experiment only).	67
5.4 Emission spectrometer (NOI-6PC) at Malaysian Institute for Nuclear Technology Research (MINT)(glasshouse experiment only).	67

5.5	Percentage of ^{15}N atom excess (a.e.) of inoculated oil palm seedlings at 130, 260 and 390 days after planting under glasshouse conditions.	69
5.6	Percentage of N_2 derived from atmosphere (Ndfa) for inoculated oil palm seedlings at 130, 260 and 390 days after planting under glasshouse conditions. (<i>Calculated based on Sp 7 k (nss) reference plants</i>)	72
5.7	Total N_2 fixed by rhizobacteria in association with oil palm seedlings at 130, 260 and 390 days after planting under glasshouse conditions. (<i>Calculated based on Sp 7 k (nss) reference plants</i>)	72
5.8	Percentage of N_2 derived from atmosphere (Ndfa) for inoculated oil palm seedlings at 130, 260 and 390 days after planting under glasshouse conditions. (<i>Calculated based on Sp 7 k (ss) reference plants</i>)	73
5.9	Total N_2 fixed by rhizobacteria in association with oil palm seedlings at 130, 260 and 390 days after planting under glasshouse conditions. (<i>Calculated based on Sp 7 k (ss) reference plants</i>)	73
5.10	Root dry weight (g plant^{-1}) of inoculated oil palm seedlings at 130, 260 and 390 days after planting under glasshouse conditions.	86
5.11	Root volume ($\text{cm}^3 \text{ plant}^{-1}$) of inoculated oil palm seedlings at 130, 260 and 390 days after planting under glasshouse conditions.	86
5.12	Primary root numbers of inoculated oil palm seedlings at 130, 260 and 390 days after planting under glasshouse conditions.	88
5.13	Top dry weight (g plant^{-1}) of inoculated oil palm seedlings at 130, 260 and 390 days after planting under glasshouse conditions.	88
5.14	Total leaf area ($\text{cm}^2 \text{ plant}^{-1}$) of inoculated oil palm seedlings at 130, 260 and 390 days after planting under glasshouse conditions.	90
5.15	Leaf chlorophyll content of inoculated oil palm seedlings at 130, 260 and 390 days after planting under glasshouse conditions.	91

5.16	Dry matter accumulation (g plant^{-1}) of inoculated oil palm seedlings at 130, 260 and 390 days after planting under glasshouse conditions.	95
5.17	Root-shoot ratio of inoculated oil palm seedlings at 130, 260 and 390 days after planting under glasshouse conditions.	95
5.18	The different leaf greenness due to inoculated treatments in the glasshouse experiment.	96
5.19	Effects of rhizobacterial inoculation on root growth and development.	96
5.20	Root dry weight (g plant^{-1}) of inoculated oil palm seedlings at 130, 260 and 390 days after planting under field conditions.	105
5.21	Root volume ($\text{cm}^3 \text{ plant}^{-1}$) of inoculated oil palm seedlings at 130, 260 and 390 days after planting under field conditions.	105
5.22	Primary root numbers of inoculated oil palm seedlings at 130, 260 and 390 days after planting under field conditions.	107
5.23	Top dry weight (g plant^{-1}) of inoculated oil palm seedlings at 130, 260 and 390 days after planting under field conditions.	107
5.24	Total leaf area ($\text{cm}^2 \text{ plant}^{-1}$) of inoculated oil palm seedlings at 130, 260 and 390 days after planting under field conditions.	109
5.25	Leaf chlorophyll content of inoculated oil palm seedlings at 130, 260 and 390 days after planting under field conditions.	109
5.26	Dry matter accumulation (g plant^{-1}) of inoculated oil palm seedlings at 130, 260 and 390 days after planting under field conditions.	113
5.27	Root-shoot ratio of inoculated oil palm seedlings at 130, 260 and 390 days after planting under field conditions.	113
5.28	Arrangements of pots in the FELDA field nursery station, Bukit Mendi Pahang.	114
6.1	N concentration and total N content (mg plant^{-1}) of inoculated oil palm plantlets after 56 days of inoculation.	134
6.2	Leaf chlorophyll content ($\text{mg chlorophyll/mg leaf fresh weight}$) of inoculated oil palm plantlets after 56 days of inoculation.	135

6.3	Primary and secondary root numbers of inoculated oil palm plantlets after 56 days of inoculation.	136
6.4	Primary root length (cm plant ⁻¹) of inoculated oil palm plantlets after 56 days of inoculation.	136
6.5	Effects of rhizobacterial inoculation on root length and formation of primary and secondary roots of tissue cultured oil palm plantlets.	137
6.6	Effects of rhizobacterial inoculation on root growth of tissue cultured oil palm plantlets at D ₀ and D ₅₆ .	137
6.7	Total dry matter (mg plant ⁻¹) of inoculated oil palm plantlets after 56 days of inoculation.	138
6.8	Colonization of rhizobacterial strains on root surface of oil palm plantlets; (a) Sp 7 k, (b) +N _i , (c) + Sp 7, (d) + CCM 3863, (e) + UPMB 10 and (f) + UPMB 13.	140

LIST OF APPENDICES

Appendix	page
1. Reagents for soil analysis.	172
2. Manuring schedule for oil palm seedlings as normally practiced by the plantation sector.	173
3. Calculation on applied $(^{15}\text{NH}_4)_2\text{SO}_4$ (10% ^{15}N a.e.) for ^{15}N isotope dilution method.	174
4. Schedule on split applications of $(^{15}\text{NH}_4)_2\text{SO}_4$ (100 mL/pot) and $(\text{NH}_4)_2\text{SO}_4$ (100 mL/pot) for separate harvests (D_{130} , D_{260} and D_{390}) of oil palm seedlings.	175
5. Reagents for electron microscopy specimen preparations (Klomprens <i>et al.</i> , 1986)	176
6. Identification of locally isolated rhizobacteria from oil palm roots by Hartmann, A. and Kirchhof, G. GSF-Research Centre, Institute of Soil Ecology, P.O. Box-1129, Oberschleissheim, Germany.	177
7. Identification test of locally isolated rhizobacteria from oil palm roots by Rene Bally, Universite Claude Bernard Lyon 1. UMR-CNRS 5557, Laboratoire d'Ecologie Microbienne, Villeurbanne Cedex, France (based on 16S rRNA sequencing).	178

